

# THE EFFECTS OF TEMPERATURE CHANGES UPON THE CHROMATOPHORES OF CRUSTACEANS

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Temperature changes as they affected the chromatophores of crustaceans were not neglected in the researches of early investigators, those of Jourdain (1878) being the first recorded in the literature to consider the possible influence of this factor. At 5°–6° C., according to his observations, the rapidity at which color changes occurred in *Nica edulis* was appreciably reduced, ceasing entirely as the temperature approached nearer to zero. At this point the animals were almost transparent, except for areas partly covered with matted white spots. Jourdain removed the eyes of those crustaceans and noted that the reddish color assumed at room temperatures, under such circumstances, disappeared entirely when the temperature of the water was lowered only to reappear again on the restoration of the temperature to its former level. Matzdorff (1883) observed no effect whatever of either high or low temperatures upon the chromatophores of *Idotea tricuspidata*. Somewhat later however, Gamble and Keeble (1900), after a few observations upon *Hippolyte varians*, reported observable color response following exposure to heat and cold. Their specimens in common with most other crustaceans possessed several differently colored pigments, reds and yellows predominating, located with one exception in discrete bodies or chromatophores. During the day the reds and yellows were usually expanded, but at night these pigments were retracted into their chromatophore centers and if it were not for a blue pigment, diffused at this time throughout the tissues and free from any chromatophore, the animals would be colorless. Gamble and Keeble selected three of these transparent blue prawns, which they called "nocturnes," and placed one in water at 15.5° C. (60° F.), one in water at 8° C. and the last in water at 32° C. (93° F.). The first animal, in reality the control as it was kept under normal temperature conditions, turned greenish-brown as was to be expected. The second one at 8° C. maintained the nocturnal blue color, showing after thirty-five minutes some traces of recovery, though one hour later this was still incomplete.

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The prawn placed in 32° C. was almost immediately killed by the heat, but remained nevertheless a brilliant nocturne for several hours, even though during the first five minutes of this experiment the temperature descended to 28° C. (83° F.).

Menke (1911) experimenting with *Idotea*, produced a contraction of the chromatophores in about 15 minutes by raising the temperature of the water from 11.5° C. to 20.5° C. This contraction was sustained for about one hour when the pigment partially re-expanded. Five and one-half hours later on, lowering the temperature to 12° C., the chromatophores again became completely expanded. But if at this time, instead of lowering the temperature of the water, it was raised to 30° C., the chromatophores also re-expanded completely. Complete expansion was also produced by lowering the temperature from 14° C. to 4° C. Doflein (1910), working with *Leander xiphias* placed several specimens in complete darkness at 5°–8° C. for two to three days. At the lapse of this time the chromatophores and the tissues of the animal were completely impregnated with blue pigment, all other pigments being completely retracted into their respective centers. But as Fuchs (1914) points out, these results might follow either from continued exposure to cold or to darkness. Megušar (1911) working with *Gelasimus*, *Potamobius*, *Palæmonetes*, and *Palæmon*, observed an expansion of the chromatophores on the sudden transfer of any of these animals from water at 16°–18° C. to water at 25°–30° C. Similarly a contraction of the chromatophores followed a sudden transfer from water at 25°–30° C. to cooler water at 16°–18° C.

The results of these experiments are admittedly confusing, though as Fuchs (1914) observed, no reasonable doubt can be entertained as to the ability of temperature changes to produce an effect of some sort upon the pigmentary responses of the crustaceans. Further investigation of the subject was thought desirable in the hope of ascertaining, if possible, just how important a factor the action of heat and cold is in determining the distribution of the chromatophore pigment of this group. For this purpose a fresh water shrimp, kindly identified for me as *Macrobrachium acanthurus* Wiegmann by Dr. W. L. Schmitt of the United States National Museum, was selected as the subject of the experiments. These shrimps were obtainable in large numbers from the Arimao river and its immediate tributaries in the vicinity of the Harvard Biological Station, Cienfuegos, Cuba. I am happy to acknowledge my thanks and appreciation to Dr. Thomas Barbour for his assistance in putting the facilities of the Harvard Cuban Station at my disposal.

When caught, the chromatophore pigments of these shrimps were

more or less extended, giving the animal a reddish-brown color. This color varied somewhat with the size of the animal, the smallest being the lightest. As collected, the shrimps ranged from 2 cm. to 10 cm. in length, measured from rostrum to telson. Males varying from 2 cm. to 3 cm. in length were selected for the experiments. Females were rejected, as at this time their abdomens were practically opaque owing to the fact that they were carrying their eggs.

A word or two regarding the color changes of *Macrobrachium* will be an aid to the understanding of what is to follow. Taken to the laboratory and placed in white glazed porcelain bowls, the shrimps in daylight soon became transparent and colorless; microscopical examination of the abdomen and telson showing the chromatophores to be completely contracted. If such animals were placed upon a black background, they assumed a dark reddish color with the chromatophores well expanded. A somewhat superficial examination disclosed the presence of two types of chromatophore pigments, both apparently located in the same chromatophore, one being reddish-brown in color and the other yellow. These facts were derived from a microscopical examination of the living pigmentary units. Detailed histological study of the chromatophores was not attempted.

Occasionally under somewhat varying conditions, animals were seen with an unmistakable bluish color observed both in the light and in the dark. The blue pigment producing this color when examined under the microscope was clearly not confined to the chromatophores, but was free in the tissues, though its concentration did appear greater about the processes of the pigmentary centers. Gamble and Keeble (1900) reported that a blue color was the regular accompaniment of the nocturnal phase of *Hippolyte varians*, a phase characterized by the retraction of all other pigments into their respective centers. According to their statement, the blue pigment in *Hippolyte* responsible for the nocturnal coloration arises as a discharge product of the chromatophores, leaving these organs on the contraction of the yellow and red pigments, and apparently being derived from them. Left free in the tissues, the blue pigment is permanently divorced from its point of origin and persists in coloring the body of the prawns until it eventually disappears.

In these experiments upon *Macrobrachium acanthurus* determinations were first made of the action of heat and cold upon the color changes in normal shrimps. The method used was as follows: Two or three animals were placed in white porcelain bowls and covered with water at room temperatures. To this was added either warm or cold water, as desired, until the particular temperature demanded by the experiment was reached. Here it was either kept constant or

altered as necessary. The responses of the shrimps to temperature changes when kept upon a black background were tested in the same manner.

Numerous experiments with normal shrimps adapted to white backgrounds demonstrated conclusively that such animals darkened when exposed to temperatures either high enough or low enough to stimulate the chromatophores. Surprising as it may seem, once the response was complete, no criteria of any sort could be established separating the darkening produced by heat from that produced by cold. The color assumed in either circumstance was a deep red-brown, while microscopical examination showed the pigments of the chromatophores to be equally well extended at high and low temperatures. The protocols of the two following experiments may be taken as typical of many others:

- 2:13—28° C. Two colorless shrimps previously kept on a white background for a day were placed in a white porcelain bowl and small pieces of ice added to the water.
- 2:16—10° C. No change in color.
- 2:18—10° C. Shrimps appear slightly reddish.
- 2:22—15° C. Shrimps somewhat darker.
- 3:00—15° C. Shrimps a pronounced brown.
- 3:25—28° C. Shrimps still brown.
- 9:30—28° C. Shrimps colorless.
  
- 2:13—28° C. Two colorless shrimps previously kept on a white background for a day were placed in a white porcelain bowl and warm water gradually-added.
- 2:16—36° C. No change in color.
- 2:18—36° C. Shrimps faintly reddish.
- 2:21—36° C. Shrimps pronouncedly brown.
- 3:00—28° C. Shrimps colorless.

In all of the experiments the appearance of the red-brown color was more rapid at high temperatures than at low. With heat only 10–15 minutes were necessary to make the animal completely dark, while with cold 30–45 minutes were required. But regardless of whether the shrimps were exposed to heat or to cold, once the point of maximal darkening was reached, the intensity of the color was equal in both cases.

When the animals were subjected to warmth the lowest temperature capable of expanding the chromatophores was found to be 35° C., while temperatures as high as 40° C. could be withstood without subsequent death, though at this temperature and slightly below it, the shrimps remained motionless, and with the exception of gill movements showed no signs of life. Therefore, within the range of 35° C. to 40° C. the color of the shrimp is determined by the temperature of its en-



vironment rather than the type of background on which it happens to be. Similarly shrimps placed in water colder than 6° C. died immediately, while any temperature above 15° C. and, of course, below 35° C., failed to produce an expansion of the chromatophore pigment. Therefore, between 6° C. and 15° C. the color of the shrimp is also determined by temperature rather than background. It might be well to mention here that the temperature of the water in which the shrimps normally lived ranged from 25° C. to 30° C.

As a check upon these results and to determine whether there was any possibility of temperature changes producing a contraction of the chromatophore pigment, experiments similar to those just described were performed upon dark shrimps while they were upon a black background. But such animals when exposed to various temperatures ranging between 6° C. and 40° C. showed no alteration whatever in the expanded condition of their chromatophores.

Recovery of normal color and activity was the rule when shrimps subjected to effective temperatures were returned to water at about 28° C. But this recovery was more rapid in shrimps treated with warmth than those treated with cold. The former required but 30 to 40 minutes, and the latter 6 to 7 hours before normal temperatures and a white background again brought their chromatophore pigment to complete contraction.

Tests were also made of the responses of blinded shrimps to temperature changes, blinding being accomplished by cutting off the eyes at the base of the eye stalk. Shortly after this operation the pigment of the chromatophores began to expand and within an hour or so, regardless of background, this expansion was complete and the animals were red-brown in color. Shrimps in this condition placed in warm and cold water and left so for an appreciable length of time—two to three hours—showed no color change of any sort. Similarly shrimps anaesthetized with 0.05 per cent chloretone, failed to show color responses to either heat or cold. Neither high nor low temperatures are then capable of exerting any contracting effect upon the pigment of the chromatophores, even when these organs are removed from the influence of any stimuli directly or indirectly produced by the retina.

As Perkins (1928) has shown in *Palæmonetes*, the withdrawal of pigment into the centers of the crustacean chromatophore is controlled by a hormone elaborated in the eye stalks, a fact which was later substantiated by Koller (1928) on *Crangon* and *Leander*. Possibly then, as temperature changes acted to expand the chromatophore pigment, there was an inhibition by heat or cold of the mechanism controlling the production of this contracting secretion. Before such

an hypothesis could be tested, it was necessary to ascertain definitely whether or not such a secretion played a part in governing the chromatic responses of *Macrobrachium*. Consequently Perkins' experiments were repeated upon this animal. Five or six shrimps were paled by placing them upon a white background for a day or more, after which their eyes were removed and thoroughly macerated in 2 cc. of 0.7 per cent NaCl. One tenth cc. of the resulting solution was then injected into the abdomens of several shrimps in the dark condition and with well expanded chromatophores. In all cases the following reactions were noted: Shortly after injection, 5–10 minutes, the shrimps began to assume a bluish color which gradually increased in intensity until within 30 minutes it had reached its maximum; this was followed by a gradual retraction of the pigment into the chromatophore centers, a retraction which persisted until the shrimps had assumed a transparent blue color. These results closely parallel the effects reported by Perkins in *Palæmonetes*, even to the formation of the blue color, and offer complete substantiation of his findings. As control experiments 0.1 cc. of the extract was injected into the abdomens of several shrimps in the light condition with no observable effect. Similarly injection of 0.1 cc. of 0.7 per cent NaCl into blinded shrimps produced no pigmentary response.

The existence of a hormone produced by the action of light upon the retina and released into the circulation to affect a contraction of the chromatophore pigment is then demonstrated in the shrimps used in these experiments. Is the formation of this hormone in any way inhibited by either high or low temperatures? Apparently not, as the following experiments show. Two sets of extracts were prepared, one from the eyes of shrimps darkened on a white background by warm water (37° C.) and the other from the eyes of shrimps darkened on a white background by cold water (15° C.), both groups being subjected to their respective temperatures for the same length of time, namely 45 minutes. Two sets of blinded shrimps were then selected, one set being injected with 0.1 cc. of one extract and the other set with the same amount of the other extract. These animals were then replaced in water at room temperature and the results noted. In both cases these darkened shrimps paled within the specified length of time, but with this difference,—the blue color previously described appeared in only one out of three of the shrimps injected with the extract prepared from the eyes of animals kept at low temperatures, while it appeared in all of the shrimps injected with the extract prepared from the eyes of animals kept at high temperatures. Neglecting for the present the significance of this variation, it is obvious that extreme temperatures

in no way inhibit the manufacture or even the potency of the chromatophore-contracting hormone elaborated by the eye stalks.

This gives us a clue as to the manner in which heat and cold affect the chromatophores of crustaceans. Unfortunately, these experiments cannot give us a conclusive solution to this problem, though the data at hand strongly indicate a direct effect. Positive information is not to be derived from experiments on limbs or bits of integument isolated from the bodies of these shrimps, as the chromatophores of such excised pieces expand at once. Consequently subjecting such preparations to temperature variations accomplished no change in the distribution of their expanded chromatophore pigment. But since experiments on blinded and chloretonized shrimps give no evidence of any other type of response to temperature changes than those seen in normal light shrimps, and since neither heat nor cold affect the secretion of the chromatophore-contracting substances elaborated in the eye stalks, it seems reasonable to assume that the responses of the chromatophore pigment of crustaceans to high and low temperatures are direct.

A word or two in regard to the blue color and its relation to temperature changes. Keeble and Gamble (1903) state that the blue color observed in nocturnal *Hippolyte* disappears completely at 60° C., while, as shown in an earlier paper (Gamble and Keeble, 1900), this color is maintained at 8° C. under conditions that in other prawns kept at a somewhat higher temperature (15° C.) produce its loss. This latter observation is in accord with the experiments of Doflein (1910) on the occurrence of a blue color in *Leander* when the animals were kept for an extended period in darkness and cold. But aside from this, it is perhaps worthy of note that, as already mentioned, blinded animals injected with the extracts prepared from the eyes of shrimps subjected to cold showed only in one third of the cases a visible blue color, whereas blinded shrimps injected with an extract from the eyes of animals kept at high temperatures never failed to become pronouncedly blue. Furthermore, throughout the course of these experiments the blue color was repeatedly observed in connection with shrimps subjected to high temperatures, while the records disclose only one instance where it was seen in connection with shrimps exposed to low temperatures; a case where an animal kept at 6° C. for about 30 minutes turned blue when returned to 28° C. Perhaps this indicates a relationship between changes in temperature and the appearance of the blue color worthy of further investigation.

A survey of the work of previous investigators dealing with the action of heat and cold upon the crustacean chromatophore reveals a wide divergence of opinion. As we have already seen, Gamble and

Keeble (1900) claimed that both high and low temperatures produce or at least maintain a retraction of the pigment, a statement with which Jourdain (1878) and Doflein (1910) are in agreement as far as low temperatures are concerned. Menke (1911), on the other hand, reports that in *Idotea* extreme high and low temperatures tend to produce an expansion of the chromatophore pigment, though moderately high temperatures (20°–25° C.) lead to a contraction. Megušar (1911), however, observed an expansion of the chromatophore pigment with heat and a contraction with cold, though this author apparently did not subject his animal to temperatures lower than 15° C. In the most recent communication Koller (1927) maintains that temperature changes have no effect whatever upon the distribution of the chromatophore pigment in *Crangon*.

The results of the present writer's investigations are more in accord with those of Menke than with those of other workers, since Menke also observed an expansion of the pigment at both ends of the effective temperature scale. *Macrobrachium acanthurus* is a semi-tropical form, habituated to water normally remaining at 25°–30° C. the year around. Therefore, the response to temperature changes of such forms might reasonably be expected to vary somewhat from those seen by Menke in *Idotea*, a form adapted to life in cooler waters. Consequently we need not be greatly concerned when *Idotea* responds to temperatures of 20°–25° C. and Cuban shrimps do not. For the latter such temperatures are obviously not warm. The important feature is that for both types an expansion of the chromatophores is produced on exposure to temperatures either extremely high or extremely low.

Among the lizards and amphibians high temperatures as a rule produce a contraction of the chromatophores and low temperatures an expansion. Among the vertebrates in general, variations from this scheme are found in certain amphibians whose chromatophores are apparently insensitive to heat and among the fishes, where innervated melanophores react to warmth by expansion and to cold by contraction. The denervated melanophores of fishes respond, however, to temperature changes as do the chromatophores of lizards and amphibians. Since in these last two groups such reactions are presumably direct, and since they are certainly direct in denervated fish melanophores, it is permissible to say that among the vertebrates the independent response of the chromatophore to heat is a contraction and to cold an expansion. In the crustacean chromatophore where there is a high probability that reactions to temperature variations are direct, though this is admittedly not certain, an expansion of the chromatophore pigment is produced both by heat and cold. On the basis of our present



knowledge then there seems to be little resemblance between the pigmentary reactions to heat and cold in the vertebrates and the crustaceans.

Among the vertebrates, especially in the lacertilians, the ability of the pigment cells to respond to temperature changes is sometimes given a thermo-regulatory significance. But the crustacean chromatophore can certainly serve no such purpose, especially as the chromatic responses of this group are controlled by factors other than heat and cold. It is inconceivable, for instance, that the form used in these experiments would ever encounter in its usual environment temperatures high enough or low enough to bring about changes in the distribution of its chromatophore pigment other than the distribution determined by background or light intensity.

#### SUMMARY

1. Expansion of the chromatophores of *Macrobrachium acanthurus*, a Cuban shrimp, follows immersion of these animals in fresh water at any temperature between 6° and 15° C. or between 35° and 40° C. This reaction occurs regardless of the background upon which the shrimp is placed. Between 15° C. and 35° C. the chromatophores of this shrimp expand when the animal is placed upon a black background and contract when the animal is placed upon a white background.

2. In blinded and chloretonized shrimps, the chromatophores are expanded and this expansion is in no way altered by changes in background or temperature.

3. Neither high nor low temperatures have any effect upon the potency or manufacture of the chromatophore-contracting substance elaborated by the eye stalks.

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